Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 8 and 19-102 are pending in the application, with claims 21, 43, 65 and 86 being the independent claims. Claims 1, 2, 4-7 and 9-18 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. Nor is the amendment admission by the Applicants of their agreement to the objections and rejections set forth by the Examiner. The amendments to the specification are to correct obvious typographical errors, to insert SEQ ID Nos, Table III labeling, and to properly capitalize trademarked names. New claims 21-102 are sought to be added. Support for this amendment can be found throughout the specification and originally-filed claims and particularly on page 7, line 13-20, page 13, line 6 and in Figure 1. Specifically, support for the range of amino acid residues 77-97 is found on page 7, lines 37 and page 13, line 6. Support for the amino acid range 74-86 is found on page 12, line 32, the ranges 25-45 and 1-444 are found in the originally-filed sequence listing, Table III, and on page 13, lines 20-21 of the specification. The nucleotide sequence range of 1-1404 is found in original claims 2 and 4. These changes are believed to introduce no new matter, and their entry is respectfully requested.

The correction to insert a "hard return" in SEQ ID NO: 9 of the Raw Sequence Listing By the Office is noted and appreciated.

Based on the above amendment and the following remarks, Applicant(s) respectfully request(s) that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Objection to the Specification

The changes to page 3, lines 15 and 16 have been made by the above amendment.

Objection to the Title

The title has been changed, at the request of the Examiner, to more accurately indicate the invention to which the claims are directed.

Objections to Claims

The objection to claims 10, 12, 15 and 18 as being substantial duplicates of claims 4-7 is respectfully traversed. However, the objection is rendered moot by the above amendment.

The objection to claim 2 as being of improper dependent form for failing to further limit the subject matter of a previous claim is respectfully traversed, but is rendered moot by the above amendment.

As all rejected claims have been cancelled, the rejections of record are believed to be rendered moot with respect to the newly-filed claims. However, the following arguments are set forth to express the position of the Applicants regarding the rejections set forth in the Action mailed 6/19/2001.

Rejections under 35 U.S.C. § 112

The rejection of claims 1, 2, 4-7 and 9-18 under 35 U.S.C. § 112, second paragraph as being indefinite for "reciting a broad limitation together with a narrow range" is respectfully traversed. However, the rejection is not believed to apply to the newly-added claims.

The rejection of claims 1, 2, 4-7 and 9-18 under 35 U.S.C. § 112, second paragraph as being indefinite because of the recitation of "a nucleotide sequence comprising nucleotide residues 1 to 1404, inclusive, as depicted in the sequence listing" is respectfully traversed. However, the rejection is not believed to apply to the newly-added claims.

The rejection of claims 1, 2, 4-7 and 9-18 under 35 U.S.C. § 112, second paragraph as being indefinite "in the recitation of "glucuronyl C-5 Epimerase, or a functional derivative thereof... ...constituted by a nucleotide sequence comprising nucleotide residues 1 to 1404" is respectfully traversed. However, the rejection is not believed to apply to the newly-added claims.

The rejection of claims 1, 4-7, 10, 12, 13, 15, 16 and 18 under 35 U.S.C. § 112, first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the relevant art that the inventor(s), at the time the invention was filed, had possession of the claimed invention" is respectfully traversed. The Examiner asserts that "The specification fails to disclose any other nucleic acids encoding a mammalian glucuronyl C5-Epimerase by any identifying characteristics or properties other than the functionality of encoding a mammalian glucuronyl C5-Epimerase." The Applicants direct the attention of the Examiner to the specification to Figure 2 and page 4, lines 6-17, where the effect of C5-Epimerase on N-deacylated, –sulfated capsular polysaccharide from *E. coli* is described, to page 4, line 35 and bridging over to page 5, line 1 where glucuronyl C5-Epimerase refers to enzymes which convert D-glucuronic acid to L-iduronic acid, to page 10, line 26 and bridging over to page 11, line 19 where the demonstration of C5-Epimerase activity and the substrate, was disclosed in detail. Therefore, contrary to the assertions of the Examiner, the specification discloses identifying characteristics and properties of the claimed

C5-Epimerase and an assay to determine the activity has been disclosed. Additionally, the specification discloses a bovine C5-Epimerase on page 5, line 32 and bridging over to page 7, line 24. Therefore, the invention as claimed has been adequately described in the specification.

Claims 1, 4-7, 10, 12, 13, 15, 16 and 18 were also rejected under 35 U.S.C. § 112, first paragraph as having a scope "not commensurate with the enablement provided by the disclosure with regard to te extremely large number of polynucleotides encoding mammalian glucuronyl C5-epimerases and derivatives thereof broadly encompassed by the claims." The Applicants respectfully directs the Examiner to the portions of the specification discussed, *supra*, in which the specification discloses detailed essays and enzyme activities of the encoded polypeptides of the claimed polynucleotides. These activities would be required for the polypeptides encoded by the claimed polynucleotides and could easily be understood and performed by one of skill in the art. The Examiner has not provided factual evidence to support his assertion that the "determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue." for the instantly-claimed polynucleotide encoding the claimed polypeptides. The Examiner has not provided any reason why one of skill in the art would not be able to make the claimed invention using the specification as filed. Therefore, the Applicants maintain that the scope of the claims encompasses all the modifications of the polynucleotides, fragments and variants claimed herein in the newly-filed claims.

Other Matters

The Applicants respectfully request clarification as to whether *all* of the priority documents have been received from the International Bureau in part 13(a) of page 1 of the Office Action Summary.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant(s) therefore respectfully request(s) that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant(s) believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Michele A. Cimbala

Michele A. Cimbala
Attorney for Applicants
Registration No. 33,851

Date: 12/19/01

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934
(202) 371-2600

Version with markings to show changes made

Claims 1, 2, 4-7 and 9-18 were cancelled, without prejudice or disclaimer.

Claims 21-102 were added.

The title was amended as follows:

DNA Sequence [Coding For] Encoding a [Mammalian] Novel Glucuronyl C5-epimerase [and a Process for its Production].

The specification was amended on page 3, with the paragraph starting on line 7 as follows:

Specific DNA sequences according to the invention are defined in the [appended claims 2, 3 and 4] Sequence Listing.

The specification was amended on page 7, lines 9-14 as follows:

The reaction is stopped by warming the mixture at 100°C for 5 minutes.

The product is purified through a [DEAE-Sephacel] DEAE-SEPHACEL column using $(NH_4)HCO_3$ 0.05 M as a buffer and eluting the product with a $(NH_4)HCO_3$ 2 M buffer.

The gathered fractions are desalted by a [Sephadex] SEPHADEX G-15 column, the fraction containing the product is lyophilized and the product is analyzed by 1H-NMR.--

The paragraph on page 5, starting at line 32 was substituted with the following paragraph:

Peptide Purification and Sequencing - The 52 kDa epimerase protein (~1 μg), purified from a detergent extract of bovine liver by chromatography on O-desulfated heparin-[Sepharose] SEPHAROSE, RED-[Sepharose] SEPHAROSE, Phenyl-[Sepharose]

SEPHAROSE, and Concanavalin A-[Sepharose] SEPHAROSE (Campbell, P., Hannesseon, H.H., Sandbäck, D., Rodén, L., Lindahl, U and Li, J-p. (1994) J Biol Chem **269**, 26953-26958), was subjected to direct N-terminal sequencing using a model 470A protein sequenator (Applied Biosystems) equipped with an on-line 120 phenylthiohydantoin analyzer (Tempst, P., and Riviere, L. (1989) Anal. Biochem. **183**, 290-300). Another sample (~1μg) was applied to preparative (12%) SDS-PAGE and was then transferred to a PVDF membrane. After staining the membrane with Coomassie Blue, the enzyme band was excised. Half of the material was submitted to direct N-terminal sequence analysis, whereas the remainder was digested with Lys-C (0.0075 U; Waco) in the presence of 1% RTX-100/10% acetonitrile/100mM Tris-HCL, pH 8.0. The generated peptides were separated on a reverse phase C4-column, eluted at a flow rate of 100 μl/min with a 6-ml 10-70% acetonitrile gradient in 0.1% trifluoroacetic acid, and detected with a 990 Waters diode-array detector. Selected peptides were then subjected to sequence analysis as described above.

The paragraph on page 6, line 19 was substituted with the following paragraph:

Probes for Screening - Total RNA was extracted from bovine liver according to the procedures of Sambrook *et al.* (1989). Single-stranded cDNA was synthesized by incubating ~5 μg of bovine liver total RNA (denatured at 65^{0}C, 3 min) with a reaction mixture containing 1 unit RNAse inhibitor (Perkin-Elmer Corp.), 1 mM of each dNTP, 5 μM random nucleotide hexamer and 1.25 units of murine leukemia virus reverse transcriptase (Perkin-Elmer Corp.) in a buffer of 10 mM Tris-HCL, pH 8.3. The mixture was kept at 42^{0}C for 45 min and then at 95^{0}C for 5 min. Degenerated oligonucleotide primers were designed based on the amino-acid sequence determined for one of the internal peptides derived from the purified epimerase

(Table 1). Single-stranded bovine liver cDNA was applied to PCR together with 100 pmols of primers 1 (sense) and 3 (antisense), in a total volume of 100 µl containing 1µl of 10% Tween 20, 6 mM $MgCl_2$, 1 mM of each dNTP, and 2.5 units [Taq] TAQ polymerase (Pharmacia Biotech) in a buffer of 10 mM Tris-HCL, pH 9.0. The reaction products were separated on a 12% polyacrylamide gel. A ~100-bp band was cut out from the gel and reamplified using the same PCR conditions. After an additional polyacrylamide gel electrophoresis, the product was isolated and sequenced, yielding a 86-bp sequence. This PCR product was subcloned into a pUC119 plasmid. The DNA fragment cleaved from the plasmid was labeled with [^{32}P] dCTP (DuPont NEN) using a Random Primed DNA Labeling Kit (Boehringer [Mannhem] Mannheim).-

The four paragraphs on page 7, starting with line 25, and ending on page 9, line 8 was substituted with the following paragraphs:

Subcloning and Sequencing of cDNA Inserts - cDNA inserts, isolated by preparative agarose gel electrophoresis (Sambrook *et al.*, 1989) after *Eco*RI restriction cleavage of recombinant bacteriophage DNA, were subcloned into a pUC119 plasmid. The complete nucleotide sequence was determined independently on both strands using the dideoxy chain termination reaction either with [^{35}S]dATP and the modified T7 polymerase ([Sequenase] SEQUENASE version 2.0 DNA Sequencing Kit; U. S. Biochemical Corp.) or the ALF™ System (Pharmacia Biotech). DNA sequences were compiled and analyzed using the DNASTAR™ program (Lasergene).

Polyclonal Antibodies and Immunodetection - A peptide corresponding to residues 77-97 of the deduced epimerase amino-acid sequence was chemically synthesized (Åke Engström,

Department of medial and Physiological Chemistry, Uppsala University, Sweden), and was then conjugated to ovalbumin using glutaraldehyde (Harlow, E. and Lane, D., (1989) in Antibodies: A Laboratory Manual, pp 78-79, Cold Spring Harbor, NY). A rabbit was immunized with the peptide conjugates together with Freund's adjuvant. After 6 boosts (each with 240 μg conjugated peptide) blood was collected and the serum recovered. The antibody fraction was further purified on a Protein A-[Sepharose] SEPHAROSE column (Pharmacia Biotech), and used for immunoblotting.

Samples of GlcA C5-epimerase were separated under denaturing conditions by 12% SDS-PAGE, and were then transferred fo a nitrocellulose membrane ([Hybond] HYBOND™ ECL). ECL immunoblotting was performed according to the protocol of the manufacturer (Amersham). Briefly, the membrane was first treated with blocking agent, then incubated with purified antibody, and finally incubated with the peroxidase labeled anti-rabbit antibody. After adding the ECL reagent, the light emitted by the chemical reaction was detected to [Hyperfilm] HYPERFILM™ ECL for 30-60 sec.

Northern Blot Hybridization - Bovine liver and lung total RNA was prepared according to Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY), and mouse mastocytoma (MCT) total RNA was extracted from a tumor cell line (Montgomery, R.I., Lidholt, K., Flay, N.W., Liang, J., Verter, B., Lindahl, U. and Esko, J.D. (1992) PNAS 89, 11327-1133[3]1) as described by Chromczynski and Sacci (1987). Total RNA from each tissue (~20 μg samples) was denatured in 50% formamide (v/v), 5% formaldehyde, 20 mM Mops buffer, pH 7.0, at 65 ^{0}C for 5 min. The denatured RNA was separated by electrophoresis in 1.2% agarose gel containing 5% formaldehyde (v/v), and was then transferred to a [Hybond]

HYBOND N^+ nylon membrane (Amersham). The RNA blot was pre-hybridized in [ExpressHyb] EXPRESSHYB Hybridization Solution (Clontech) at 65 ^{0}C for 1 h, and subsequently hybridized in the same solution with[-]a [^{32}P]dCTP-labeled DNA probe (a 2460 bp fragment including the 5'-end of the cDNA clone; see the sequence listing). The membrane was washed in 2x SSC, 0.5% SDS at the same temperature for 2 x 15 min and in 0.5x SSC, 0.5% SDS for 2 x 5 min. The membrane was exposed to Kodak X-ray film at -70^{0}C for 24 h.

The specification was amended on page 12, with the paragraphs starting with line 19 as follows:

Characterization of cDNA and predicted protein structure - The total cDNA sequence identified, in all 3085 bp, contains an open reading frame corresponding to 444 amino-acid residues [the sequence listing] (SEQ ID NO: 13). Notably, the coding region (1332 bp) is heavily shifted toward the 5'-end of the available cDNA, and is flanked toward the 3'-end by a larger (1681 bp) noncoding segment. The deduced amino-acid sequence corresponds to a 49,905 dalton polypeptide. All of the five peptides isolated after endo-peptidase digestion (Table I) were recognized in the primary structure deduced from the cDNA [the sequence listing] (SEQ ID NO: 12). One of these peptides (peptide 1) is identical to the N-terminus of the isolated liver protein. This peptide was found to match residues 74-86 of the deduced polypeptide sequence (SEQ ID NO: 13). The enzyme isolated from bovine liver thus represents a truncated form of the native protein.

Generation of mRNA from an expression vector inserted with the 3-kb cDNA clone, followed by incubation of the product with rabbit reticulocyte lysate in the presence of [^{35}S] methionine, resulted in the formation of a distinct labeled protein with an estimated M_r of ~50

kDa (Fig. 1). This product was recognized in immunoblotting (data not shown) by polyclonal antibodies raised against a synthetic peptide corresponding to residues 77-79 [see the sequence listing] (SEQ ID NO: 13) of the deduced amino-acid sequence. The same antibodies also reacted with the isolated ~52 kDa bovine liver protein (data not shown). These observations establish that the 3-kb cDNA is derived from the transcript that encodes the isolated ~52 kDa bovine liver protein.

The paragraph on page 13, starting with line 13, was substituted with the following paragraph:

The cDNA structure indicates the occurrence of 3 potential N-glycosylation sites ([the sequence listing] Table III). Sugar substituents may be important for the proper folding and catalytic activity of the enzyme, since the protein expressed in bacteria (which also gave a strong Western signal towards the polyclonal antibodies raised against the synthetic peptide; data not shown) was devoid of enzymatic activity. A potential transmembrane region is underlined in [the sequence listing] Table III. The predicted protein contains two cystein residues, only one of which occurs in the isolated (truncated) protein. Since NEM was inhibitory to epimerase activity (data not shown), this single cystein unit may be essential to the catalytic mechanism.

On page 18, line 1, "SEQUENCE LISTING" was deleted and substituted with the following:

Table III

P:\USERS\Mtung\1708\038\faom